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Practical Applications of Paper Electrophoresis

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• Paper electrophoresis of proteins is a simple, economical method well adapted to routine laboratory use. It can give important diagnostic information concerning serum proteins, and is invaluable in the differential diagnosis of diseases in which there are abnormal hemoglobins.

ANIMAL PROTEINS may be separated and characterized by a variety of methods ranging in complexity from simple salting-out procedures to the elaborate differential sedimentation obtained from ultracentrifugation. One method of protein separation, electrophoresis, is based upon the different ionic charges carried by various proteins at a given pH. If a solution of mixed proteins is placed in an electrical field, different groups of these proteins will show characteristic migration patterns, and may thus be separated and identified.

The clinical value of serum protein electrophoresis has been long known, and has been reviewed in detail by Luetscher,[11] Gutman,[6] and Fisher.[4] However, broad application of this technique has been hampered by the expense and complexity of the equipment necessary for its performance. In 1950 Durrum[1] described an apparatus by which electrophoretic separation of proteins could be accomplished on filter paper. This apparatus, simple and inexpensive in design, can be purchased from commercial sources, or can be readily constructed from available laboratory equipment. Two different types of apparatus are now in use: the original Durrum design in which the paper strips are suspended in air over a glass rod (Figure 1); and the modification suggested by Kunkel and Tiselius[9] in which sheets of filter paper are held between silicone-coated glass plates. Both methods are well adapted to routine use by a clinical laboratory.

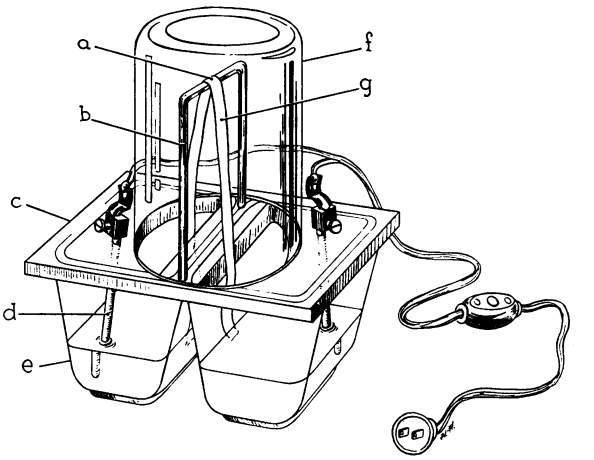

Figure 1.—Paper electrophoresis apparatus. (a) Point of application of test solution; (b) glass rod; (c) plastic cover; (d) carbon electrode; (e) glass basin containing buffer; (f) glass cover; (g) filter paper strip. (Reprinted from Spaet,[14] through the courtesy of the *Journal of Laboratory and Clinical Medicine.*)

METHODS AND MATERIALS

Both the Durrum and Kunkel-Tiselius types of paper electrophoretic apparatus were used in the present study. Detailed descriptions of these techniques have been published elsewhere.[1, 9]

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In all studies, the apparatus used was charged with veronal buffer, pH 8.6. For the Durrum apparatus the electricity was 110-volt direct current taken directly from the hospital line. At this potential, adequate separation of serum proteins was obtained in 12 to 16 hours; separation of hemoglobins took 24 hours. Higher voltage can be used with the Kunkel-Tiselius apparatus, as evaporation is no problem. Radio "B" batteries connected in series, or a home-made high voltage transformer supplies 810 to 1,200 volts of potential, and runs can be accomplished in two to five hours.

For serum protein studies, undiluted serum was used without modification. Hemoglobin was rendered stroma-free and dissolved in buffer according to methods previously described.[14] Three lambda (three divisions on a pipette for counting erythrocytes) of test fluid were used. After separation, all strips were stained with bromphenol blue according to the method of Kunkel and Tiselius,[9] except that, instead of drying, they were "fixed" prior to staining by immersion in a solution of 10 per cent $HgCl_2$ in 95 per cent ethanol for five minutes.

RESULTS

Serum Proteins: As can be seen in Figure 2, at least five serum protein components can be resolved by paper electrophoresis. Fortunately, bromphenol blue combines stoichiometrically with the serum proteins so that the density of staining is proportional to the concentration of protein component. Thus it is possible to detect both the presence of abnormal proteins and deviations from the normal concentration of physiological proteins. In Figure 2 a normal pattern is shown together with patterns in three specimens of blood from patients with multiple myeloma. Each patient has an abnormal band: one at the β_2 position; one at the γ position; and one which migrates even more toward the cathode than any normal γ globulin. Such patterns may be virtually diagnostic of multiple myeloma when other studies are inconclusive.

Figure 3 shows serial patterns obtained from a 15-year-old boy with the nephrotic syndrome. This disorder resolved following cortisone therapy. The initial pattern shows protein changes which are almost peculiar to this disease, namely: diminished albumin and γ globulin combined with increased α_2 and β globulins. The succeeding patterns reflect the clinical improvement, with restoration of the protein fractions to an almost normal picture. A protein spot seen at the origin represents uncoagulated fibrinogen. This protein persisted even after apparently complete clotting in this patient, who had severe hemophilia.

Numerous other electrophoretic abnormalities of

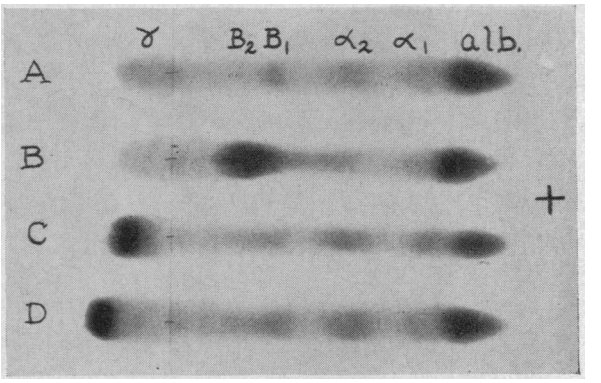

Figure 2.—Electrophoretic patterns of patients with multiple myeloma. (*A*) Normal control; (*B*) abnormal protein migrating as a β_2 globulin; (*C*) abnormal γ component; (*D*) abnormal protein which moves farther toward the cathode than any normal component.

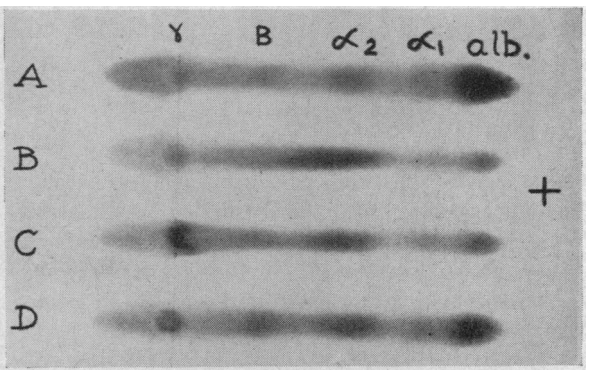

Figure 3.—Sera from a patient with the nephrotic syndrome. (*A*) Normal control; (*B*) patient prior to therapy; (*C*) one month following cortisone; (*D*) two months following cortisone. Note that initially there is a severe hypoalbuminemia together with increased α_2 and β globulins. A partial restoration toward a normal pattern is seen following therapy. (These sera were provided through the courtesy of Dr. Quentin Deming.)

the serum are found in disease, and these are readily demonstrated by the present technique. (There is not space here for an atlas of these patterns. A more detailed discussion of this subject is to be found in Luetscher's review,[11] and in Gutman's monograph.[6])

Hemoglobin: Electrophoretic studies of hemoglobin became of clinical importance in 1949 when Pauling[13] discovered different migration properties between normal hemoglobin and that of patients with sickle cell anemia. Since that time three additional electrophoretically abnormal hemoglobins have been discovered, each associated with a different morphologic pattern of erythrocytes and a different set of clinical manifestations. Each abnormality of hemoglobin appears to be transmitted according to Mendelian laws. If any pigment is "heterozygous," so that it is combined with normal hemoglobin, the patient has the respective "trait" and is free of clinical findings. Any combination of abnormal pig-

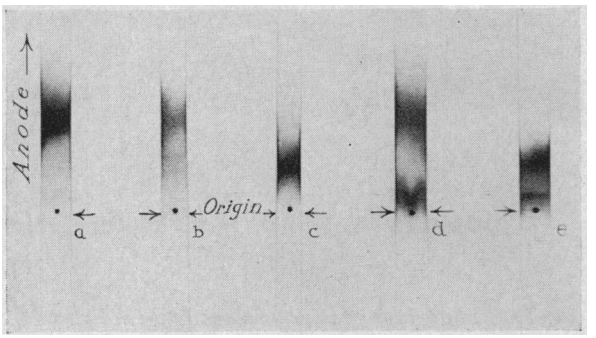

Figure 4.—Electrophoretic patterns of normal and abnormal hemoglobins. (a) Normal; (b) sickle cell trait; (c) sickle cell anemia; (d) type "c" trait; (e) combined sickle and "c" pigments. (Reprinted from Spaet[12] through the courtesy of the *Journal of Laboratory and Clinical Medicine.*)

ments, either "homozygous" or "heterozygous," appears to be associated with a hemolytic process. Sickle cell anemia is a homozygous combination of the sickle type pigment; sickle cell trait represents a heterozygous combination of normal and sickle pigments. Itano recently published an excellent review of this subject.[7]

Figure 4 shows the paper electrophoretic patterns obtained from patients with various combinations of abnormal hemoglobins. "Homozygous" and "heterozygous" combinations are clearly demonstrated. To the author the method has proved most valuable in differentiating between sickle cell anemia, and sickle cell trait with intercurrent anemia. It has also made possible the detection of cases with some of the more recently described pigments—cases that had formerly presented diagnostic problems. In one such case the patient proved to have the first reported "homozygous" type "c" hemoglobin.[16]

DISCUSSION

Electrophoresis on filter paper as described is a procedure well adapted to routine laboratory use. The cost is negligible, the technique can be learned by unskilled personnel in a short time, and many determinations may be run simultaneously. The information gained from electrophoretic studies of the serum proteins exceeds that obtained from the usual protein analyses, and the method is less laborious. It is especially adapted to pediatric needs. Since only minute amounts of blood are needed, capillary blood can be used. In the study of hemoglobin, the information provided by electrophoretic analysis cannot be duplicated by other methods.

A modification of the present technique can provide quantitation of the electrophoretically separated protein fractions. Three methods are available; the bromphenol blue may be eluted from serial segments of the paper with sodium hydroxide and measured in a spectrophotometer, according to the procedure of Kunkel and Tiselius;[9] or similar segments may be subjected to analysis by a microkjeldahl method;[10] and most recently a photoelectric scanning device has been constructed by which the density of bromphenol blue deposited in the paper is read directly.[5] Any of these methods gives reproducible results, which, however, are not strictly comparable to those obtained by older techniques.

In addition to its value as a clinical tool, paper electrophoresis has numerous research applications. Special stains have been used to measure lipoproteins, and valuable information has been obtained concerning patterns in diseases involving abnormal lipid metabolism.[3, 8] Specific serum components such as antibodies have been separated and characterized.[12, 15] A special modification of the method has provided a technique by which serum protein components may be fractionated electrophoretically in quantity.[2] These are a few of the applications which demonstrate the extreme versatility of paper electrophoresis.

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